REMARKS

Applicants submit this amendment to insert the required references to SEQ ID NOS of the Sequence Listing filed concurrently herewith and to indicate the insertion point for the Sequence Listing. Please insert the Sequence Listing filed concurrently herewith following the abstract, and renumber pages 1-11 of the Sequence Listing as pages 41-51.

In responding to the Notice, no new matter has been added.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date 1 July 2003

By _____

Foley & Lardner
3000 K Street, N.W., Suite 500
Washington, D.C. 20007
Telephone: 202-672-5300
Facsimile: 202-672-5399

George C. Best
Attorney for Applicants
Registration No. 42,322

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No.19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

MARKED UP VERSION TO SHOW CHANGES MADE

Marked up v rsion of paragraph [0011] is below:

[0011] Particularly preferred procytotoxins have the following structures: (1) Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Gly-Ala-Ile-Gly-Gln-Pro]-(X) **(SEQ ID NOS 1 & 2, respectively)**, and (2) Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln- Ser-Ser-Phe(or Tyr)-Tyr-Ser-Gly(or Ser)]-(X) **(SEQ ID NOS 3 & 4, respectively)**, wherein (X) is the inactivator as described herein and the peptide marked in brackets can be oriented in either direction. The inactivator, for example, can be a microbead, amino acid, peptide, phage, or phage filament. Preferably, the procytotoxin further contains a targeting molecule. Still preferred, the targeting molecule is a neovascular targeting sequence of an anti-fibronectin ED-B antibody. Also preferred, the targeting molecule is an RGD targeting sequence.

Marked up version of paragraph [0012] is below:

[0012] Other preferred procytotoxins further are charge neutralized, in addition to steric determinants. For example, Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys(R)-Arg-Lys(R)-Arg-Gln-[Gln-Gly-Ala-Ile-Gly-Gln-Pro]-(X) **(SEQ ID NOS 21& 22, respectively)**, wherein (X) is the inactivator as described herein, the peptide marked in brackets can be oriented in either direction, and wherein R is independently selected from the group consisting of the [unmodified ε-amino group of the adjacent lysine residue], [ε-γ]-Glu, [ε-γ]-Glu-[α-γ]-$(Glu)_{1-3}$, [ε-α]-$(Phe)_{1-3}$, [ε-α]-$(Tyr)_{1-3}$, [ε-α]-$(Trp)_{1-3}$, [ε-α]-$(Lys)_{1-3}$ and [ε-α]-$(Arg)_{1-3}$, wherein [ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [ε-α] represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

Marked up version of Table 1, on pag 8, is below:

TABLE 1 Amino Acid Sequence of Selected Cytolytic Peptides

Amoebapore Helix 3 (*Entamoeba histolytica*)

NH_2-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-**[COOH]**$\underline{CONH_2}$ **(SEQ ID NO: 5)**

Cecropin A (*Antheria pernyi*)

NH_2-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Asn-Ile-Arg-Asp-Gly-Ile-Ile-Lys-Ala-Gly- Pro-Ala-Val-Ala-Val-Val-Gly-Gln-Ala-Thr-Gln-Ile-Ala-Lys-COOH **(SEQ ID NO: 6)**

Cecropin B (*Antheria pernyi*)

NH_2-Lys-Trp-Lys-Ile-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Arg-Asn-Ile-Arg-Asn-Gly-Ile-Ile-Lys-Ala-Gly-Pro-Ala-Val-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu-COOH **(SEQ ID NO: 7)**

Cecropin D (*Antheria pernyi*)

NH_2-Trp-Asn-Pro-Phe-Lys-Glu-Leu-Glu-Lys-Val-Gly-Gln-Arg-Val-Arg-Asp-Ala-Val-Ile-Ser-Ala-Gly-Pro-Ala-Val-Ala-Thr-Val-Ala-Gln-Ala-Thr-Ala-Leu-Ala-Lys-COOH **(SEQ ID NO: 8)**

Melittin (*Apis mellifera*)

NH_2-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-COOH **(SEQ ID NO: 9)**

Marked up version of paragraph [0025] is below:

[0025] Three isoforms of amoebapore are known: amoebapore A, B and C, respectively. This peptide is stabilized by three disulfide bonds and contains four mostly amphipathic alpha-helical structures. The third amphipathic helical structure (helix 3) retains the cytolytic activity similar to the wild type peptide. A synthetic peptide based on the sequence of its third amphipathic alpha helix have recently been shown to have cytolytic activity for nucleated cells at high concentrations (10-100 μM) (Leippe *et al.*, (1994) Proc. Natl. Acad. Sci. USA 91: 2602). Accordingly, a particularly preferred cytotoxin is **a derivative of the** **[an]** amoebapore **[derivative]** **cytolytic peptide** listed in Table I: NH_2-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-**[COOH]**$CONH_2$ **(SEQ ID NO: 5)**.

Marked up version of paragraph [0053] is below:

[0053] For instance, the procytotoxin of the present invention comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Gly-Ala-Ile-Gly-Gln-Pro]-(X) **(SEQ ID NOS 1 & 2, respectively)**, wherein (X) is an inactivator and the peptide marked in brackets can be oriented in either direction. The inactivator can be selected from the group consisting of a microbead, an amino acid, a peptide, phage and a phage filament. Cleavage by MMP at this peptide will yield a melittin peptide with few additional amino acids on the C-terminus (Gly-Ala-Ile) which should not interfere with pore formation.

Marked up version of paragraph [0054] is below:

[0054] In a related vein, the procytotoxin of the present invention comprises Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln- Ser-Ser-Phe(or Tyr)-Tyr-Ser-Gly(or Ser)]-(X) **(SEQ ID NOS 3 & 4, respectively)**, wherein (X) is an inactivator and the peptide

Marked up versi n of paragraph [0055] is below:

[0055] Also contemplated in the instant invention is a targeting molecule that adds an additional measure of selectivity. For example, the procytotoxin may comprise the following structure: lytic peptide-[Gln-Gly-Ala-Ile-Gly-Gln-Pro]-Lys-[ε-γ]-biotin- **(SEQ ID NO: 10)** streptavidin coated microbead-RGD targeting sequence.

Marked up version of paragraph [0060] is below:

[0060] Particularly preferred procytotoxins include amoebapore, its analogs and its derivatives that contains one or more γ-linked glutamate residues linked via a peptide bond to the epsilon amino group of at least one lysine, preferably the C-terminal-most lysine (hereinafter "γ-glutamate-masked amoebapore analog"). A particularly preferred procytotoxin has the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-[ε-γ]-Glu-[α-γ]-Glu **(SEQ ID NO: 11)**, wherein [ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate and [α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate.

Marked up version of paragraph [0061] is below:

[0061] In addition, amoebapore and other cytotoxic peptides can be modified with other amino acids. One such exemplary protoxin has the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-[ε-α]-Phe **(SEQ ID NO: 12)**, wherein [ε-α] represents a peptide bond between the epsilon amino group of lysine and the alpha carboxyl group of the adjacent phenylalanine. Another exemplary protoxin that can be activated by chymotrypsin-like activity has the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys([ε-α]-Phe)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-[ε-α]-Phe **(SEQ ID NO:**

13), using the same nomenclature and where Lys([ε-α]-Phe)-Leu represents a linkage between the epsilon amino group of lysine and the alpha carboxy group of phenylalanine, and a standard peptide linkage between lysine and phenlyalanine. Of course, the phenylalanine may be replaced with other amino acids, such as tyrosine and tryptophan in the case of chymotrypsin-like activity. In some instances, in order to invoke trypsin-like activity, it may be beneficial to utilize positively charged amino acids, like arginine and lysine, instead of phenylalanine.

Marked up version of paragraph [0062] is below:

[0062] Other particularly preferred procytotoxins include melittin, its analogs and its derivatives that contain at least one γ-linked glutamate residue linked via a peptide bond to the epsilon amino group of a lysine (hereinafter "γ-glutamate-masked melittin analog"). As indicated in Table 1, melittin has two lysines and two adjacent arginines near its C-terminus. When one of the lysines is so masked, it is expected that the free alpha carboxyl group would act to neutralize the adjacent arginine, further contributing to the inhibition of the toxic activity of melittin. A particularly preferred procytotoxin has the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys([ε-γ]-Glu)-Arg-Lys([ε-γ]-Glu)-Arg-Gln-Gln **(SEQ ID NO: 14)**, wherein -Lys-([ε-γ]-Glu)-Arg- represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate and a standard peptide bond between the lysine and arginine residues. Of course, -Lys-([ε-γ]-Glu)-Arg- can be replaced, for example, by -Lys([ε-α]-Phe)-Leu-, as detailed above, and phenylalanine can be replaced by other amino acids like tyrosine and tryptophan to invoke chymotrypsin-like activity. In some instances, when trypsin-like activity is being invoked, it may be beneficial to utilize positively charged amino acids, like arginine and lysine, instead of phenylalanine in this latter example.

Marked up version of paragraph [0064] is below:

[0064] A set of particularly preferred procytotoxins have the following structures: (1) Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys(R)-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys(R)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys(R) **(SEQ ID NO: 15)**, and (2) Gly- Ile-Gly-Ala-Val-Leu-Lys(R)-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys(R)-Arg-Lys(R)-Arg-Gln-Gln **(SEQ ID NO: 16)**, wherein R is independently selected from the group consisting of the ε-amino group of the adjacent lysine residue, [ε-γ]-Glu, [ε-γ]-Glu-[α-γ]-$(Glu)_{1-3}$, [ε-α]-$(Phe)_{1-3}$, [ε-α]-$(Tyr)_{1-3}$, [ε-α]-$(Trp)_{1-3}$, [ε-α]-$(Lys)_{1-3}$ and [ε-α]-$(Arg)_{1-3}$, wherein [ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [ε-α] represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds. With regard to the subscripted numbers, it is understood that larger numbers of amino acids are possible, *e.g.*, 4, 5, 6, *etc.*, but 1, 2, and 3 are anticipated to be optimal.

Marked up version of paragraph [0092] is below:

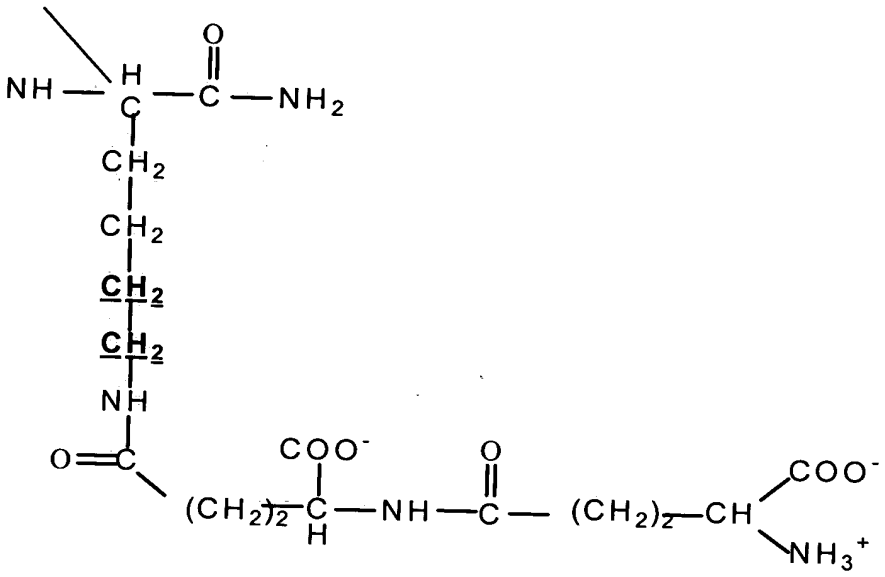
[0092] Below is shown a diagram of the initial cytolytic peptide and the procytolytic peptide synthesized by the addition of the two γ glutamate linked side-chain glutamic acid residue to the ε amino group of the C-terminal lysine.

Cytolytic Peptide: (SEQ ID NO: 17)

N-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-[Leu]-Ile-Gln-Leu-Ile-Glu-Asp-Lys-**[COOH]$CONH_2$**

Procytolytic Peptide: (SEQ ID NO: 18)

N-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-[L u]-Ile-Gln-Leu-Ile-Glu-Asp—



Marked up version of paragraph [0097] is below:

[0097] This example demonstrates that the inventive γ-glutamate-masked cytolytic peptides have specificity for cancer cells other than those expressing PSMA. This experiment, utilized a melittin analog having A [ε-γ]-Glu-[α-γ]-Glu at each of lysines 21 and 23: NH_2-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys([ε-γ]-Glu-[α-γ]-Glu)-Arg-Lys([ε-γ]-Glu-[α-γ]-Glu)-Arg-Gln-Gln-COOH **(SEQ ID NO: 19)** Two prostate tumors (PNCap and DU0145), two ovarian tumors (HeLa and SK-OV-3), one lung tumor (LLC1) and one melanoma (B16) were tested. Cultured cells were treated with 1, 10, 50 or 100 μM peptide. Results, depicted in Figure 4, show strong lytic activity against all tumors.

Marked up version of paragraph [0102] is below:

[0102] As an example, melittin may be inactivated by attaching a phage filament, other peptide, or a biotin-streptavidin microbead, according to the following: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Gly-Ala-Ile*Gly-Gln-Pro]-(X) **(SEQ ID NOS 1 & 2, respectively)**, wherein * denotes an MMP cleavage site and (X) is an inactivator. MMP cleavage will yield a melittin peptide with three additional amino acids on the C-terminus (Gly-Ala-Ile), which should not interfere with pore formation.

Marked up version of paragraph [0104] is below:

[0104] The procytotoxin may further comprise a targeting sequence, such as Biotin-Gly-Gly-Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys **(SEQ ID NO: 20)** (RGD-4C) $\alpha_v\beta_3$ integrin targeting peptide or biotin-anti-fibronectin ED-B antibody.

In the Claims:

Please amend the claims as follows:

15. (Amended) The procytotoxin of claim 14, wherein said cytolytic peptide comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Gly-Ala-Ile-Gly-Gln-Pro] **(residues 1-32 of SEQ ID NOS 1 or 2)**.

18. (Amended) The procytotoxin of claim 14, wherein said cytolytic peptide comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln- Ser-Ser-Phe(or Tyr)-Tyr-Ser-Gly(or Ser)] **(residues 1-32 of SEQ ID NOS 3 or 4)**.

36. (Amended) The method of claim 35, wherein said cytolytic peptide comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-

Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Gly-Ala-Ile-Gly-Gln-Pro] **(residues 1-32 of SEQ ID NOS 1 r 2)**.

38. (Amended) The method of claim 35, wherein said cytolytic peptide comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Ser-Ser-Phe(or Tyr)-Tyr-Ser-Gly(or Ser)] **(residues 1-32 of SEQ ID NOS 3 or 4)**.

54. (Amended) The method of claim 53, wherein said cytolytic peptide comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln-Gly-Ala-Ile-Gly-Gln-Pro] **(residues 1-32 of SEQ ID NOS 1 or 2)**.

57. (Amended) The method of claim 53, wherein said cytolytic peptide comprises the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-[Gln- Ser-Ser-Phe(or Tyr)-Tyr-Ser-Gly(or Ser)] **(residues 1-32 of SEQ ID NOS 3 or 4)**.